

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application.

Please amend claims 1-21 as follows and cancel claim 22:

Listing of Claims:

Claim 1 (currently amended): A method for the quantitative evaluation of a rearrangement or of a targeted genetic recombination of an individual, which method ~~is characterized in that it~~ comprises at least:

- (a) the extraction of human genomic DNA from a biological sample,
 - (b) the amplification of a segment of said genomic DNA, between a few hundred base pairs and several tens of kb in size, by multiplex PCR, in the presence:
 - of one or more pairs of primers, selected so as to correspond to the following characteristics:
 - at least one of said primers of one of said pairs of primers hybridizes upstream and/or at the 5' end of a Vx gene to be amplified, which may be involved in said genetic rearrangement;
 - at least the other of said primers of one of said pairs of primers hybridizes downstream and/or at the 3' end of a Jy gene to be amplified, which may be involved in said genetic rearrangement;
 - and of a DNA polymerase or a mixture of DNA polymerases for amplifying genomic DNA segments between a few hundred base pairs and several tens of kb in size, preferably greater than 10 kb in size, and having a correction activity that makes it possible to substantially improve the elongation;
- said amplification comprising, in addition to the initial denaturation step, cycles of denaturation, hybridization and elongation, in which the elongation steps are carried out at least for 10 minutes at 68°C-72°C;
- c) the separation of the gDNA fragments amplified, and

d) the detection of the rearranged or recombined segments.

Claim 2 (currently amended): The method for the quantitative evaluation of the immune repertoire of an individual by genetic rearrangement as claimed in claim 1, comprising ~~characterized in that it comprises:~~

a) the extraction of human genomic DNA from a biological sample,
b) the amplification of a segment of said genomic DNA, between a few hundred base pairs and several tens of kb in size, by multiplex long PCR, in the presence:

of one or more pairs of primers, selected so as to correspond to the following characteristics:

- at least one of said primers of one of said pairs of primers, called primer V, hybridizes specifically with a region located upstream of the RSS sequence of a V α gene to be amplified, corresponding to a V segment of the variable domain of the α chain of a T-cell receptor (TCRAD);

- at least one of said primers of one of said pairs of primers, called primer J, hybridizes specifically with a region located downstream of the RSS sequence of a J γ gene to be amplified, with the 3' end of said J γ gene to be amplified or in said J γ gene to be amplified, corresponding to a J segment of the α chain of a T-cell receptor;

and of a DNA polymerase or a mixture of DNA polymerases for amplifying genomic DNA segments between a few hundred base pairs and several tens of kb in size, and having a correction activity that makes it possible to substantially improve the elongation;
said amplification comprising, in addition to the initial denaturation step, cycles of denaturation, hybridization and elongation, in which the elongation steps are carried out at least for 10 minutes at 68°C-72°C;

(c) the separation of the gDNA fragments amplified, and
(d) the detection of the recombined V(D)J segments.

Claim 3 (currently amended): The method as claimed in claim 1 ~~or claim 2, characterized in that, wherein~~ in the amplification step (b), the selection of the primers is carried out:

- by systematic analysis of the entire locus concerned, and in particular of the human TCRAD locus, using a suitable software,
- selection of the primers whose 3'OH end is complementary only to the region of interest,
- elimination of the primers forming autodimers or stable hairpins, in particular by analysis with a suitable software, and
- elimination of the pairs of primers which form hybrids with one another.

Claim 4 (currently amended): The method as claimed in claim 3, ~~characterized in that wherein~~ the primers V and J of the pairs of primers V/J are selected from the group consisting of the primers of sequences SEQ ID NO: 1-21.

Claim 5 (currently amended): The method as claimed in claim 1 ~~any one of claims 1 to 4, characterized in that wherein~~ the amplification step (b) advantageously uses additional primers for amplifying, in addition, at least one of the following segments: D segments, V segments and J segments of the TCR β , γ , δ chains and, optionally, segments of the immunoglobulin chains.

Claim 6 (currently amended): The method as claimed in claim 1 ~~any one of claims 1 to 5, characterized in that, wherein~~ in the amplification step (b), the multiplex long PCR (LPCR) reaction is carried out after purification of the DNA, or directly on a cell lysate.

Claim 7 (currently amended): The method as claimed in claim 1 ~~any one of claims 1 to 6, characterized in that, wherein~~ in the amplification step (b), the elongation steps are incremented by 15-20 seconds per additional elongation cycle.

Claim 8 (currently amended): The method as claimed in claim 1 ~~any one of claims 1 to 7,~~
~~characterized in that~~ wherein step (c) consisting of separation of the amplified DNA fragments is
carried out by electrophoretic migration on a gel, preferably pulsed-field migration.

Claim 9 (currently amended): The method as claimed in claim 1 ~~any one of claims 1 to 7,~~
~~characterized in that~~ wherein step (c) consisting of separation of the amplified DNA fragments is
carried out by microcapillary separation.

Claim 10 (currently amended): The method as claimed in claim 1 ~~any one of claims 1 to 9,~~
~~characterized in that~~ wherein the detection step (d) can advantageously be carried out by
Southern transfer of the amplified products onto nylon membranes, followed by visualization
after hybridization with one or more nucleotide probes labeled with a radioactive isotope or a
fluorochrome.

Claim 11 (currently amended): The method as claimed in claim 10, ~~characterized in that~~ wherein
the probes are advantageously selected from the group consisting of the sequences SEQ ID NO:
22-37.

Claim 12 (currently amended): The method as claimed in claim 1 ~~any one of claims 1 to 9,~~
~~characterized in that~~ wherein the detection step (d) can advantageously be carried out by using a
labeled base (labeled with a radioactive isotope or a fluorochrome) during the amplification, and
then by measuring the incorporation thereof directly in the gel.

Claim 13 (currently amended): The method as claimed in claim 1 ~~any one of claims 1 to 9,~~
~~characterized in that~~ wherein the detection step (d) can advantageously be carried out by using a
DNA-labeling agent during the migration, and detecting after excitation in the UV range or at
another appropriate wavelength.

Claim 14 (currently amended): The method as claimed in claim 1 ~~any one of claims 1 to 9,~~
~~characterized in that~~ wherein the detection step (d) can advantageously be carried out by using
primers labeled with fluorochromes or other enzymatic revealing means during the amplification.

Claim 15 (currently amended): A method for the follow-up to a treatment for a pathology in
which the immune repertoire is initially modified, in an individual in need thereof ~~concerned~~,
which method comprises ~~is characterized~~:

- ~~in that it implements~~ implementing the method for the evaluation of the immune
repertoire, as claimed in claim 1 ~~any one of claims 1 to 14~~, at the beginning of treatment,

- ~~in that said evaluation method is reiterated~~ reiterating said evaluation method at various
phases of the treatment, and

- ~~in that the profile of the immune repertoire obtained each time is compared~~ comparing
the profile of the immune repertoire obtained each time with that of a standard immune
repertoire, in order to evaluate the response of said individual to said treatment.

Claim 16 (currently amended): A method for the measurement of the antigen receptor repertoire
during the various phases of a pathology in which the immune repertoire is modified, in an
individual in need thereof ~~concerned~~, which method comprises ~~is characterized~~:

- ~~in that it implements~~ implementing the method for the evaluation of the immune
repertoire, as claimed in claim 1 ~~any one of claims 1 to 14~~, at various phases of the pathology,
and

- ~~in that the profile of the immune repertoire obtained each time is compared~~ comparing
the profile of the immune repertoire obtained each time with that of a standard immune
repertoire, in order to evaluate the evolution of said pathology.

Claim 17 (currently amended): The method as claimed in claim 1 ~~any one of claims 1 to 16,~~
~~characterized in that~~ wherein the biological sample consists of T lymphocytes of any origin.

Claim 18 (currently amended): The method as claimed in claim 17, ~~characterized in that~~ wherein said T lymphocytes are selected from the group consisting of thymic cells, of T lymphocytes from peripheral blood, of T lymphocytes from other lymphoid organs, of T lymphocytes from various organs and of T lymphocytes derived from tumors or from inflammatory sites.

Claim 19 (currently amended): A kit for the quantitative evaluation of the immune repertoire of an individual comprising, ~~characterized in that it comprises~~, in addition to the usual buffers and reagents for carrying out a PCR, primers and probes corresponding to the sequences SEQ ID NO: 1-37 as defined in claims 4 and 11.

Claim 20 (currently amended): A primer ~~that can be used in a method as claimed in any one of claims 1 to 18, characterized in that it is~~ selected from the group consisting of the oligonucleotide primers corresponding to the sequences SEQ ID NO:1-21.

Claim 21 (currently amended): A detection probe ~~that can be used in a method as claimed in any one of claims 1 to 18, characterized in that it is~~ selected from the group consisting of the oligonucleotide probes of sequences SEQ ID NO: 22-37.

Claim 22 (cancelled)